

# The Effect of Fish Oil Fatty Acid Supplementation on Two-Step Tuberculin Skin Test: A Randomized Controlled Clinical Trial

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Received 2016 June 12; Revised 2017 January 02; Accepted 2017 January 23.

## Abstract

**Background:** A two-step tuberculin skin test (TST) should be performed if the subsequent TSTs are to be conducted at regular intervals, especially among health care professionals, and its interpretation of serial TST is particularly complex.

**Methods:** In this randomized controlled clinical trial, the effect of fish oil fatty acid (FA) supplementation on two-step TST was investigated. The outcome was considered a change in 2 sequential TST induration sizes; 6 mm or more were considered as a criterion to distinguish the increase in reaction size. The study protocol was approved in the ethic committee of Qazvin University of Medical Sciences. In the present study, 213 healthy participants (medical and nursing students of Qazvin University of Medical Sciences in 2014) were randomly assigned into a normal diet (control) or fish oil supplementation (treatment) group. One gram of fish oil supplement was given to the treatment group daily for 5 consecutive days.

**Results:** The mean secondary induration and the difference between the primary and secondary induration sizes were significantly higher in the fish oil users ( $P < 0.001$ ). Noticeable increase in TST size in the treatment group was significantly higher than in the control (16.3% vs. 1.2%).

**Conclusions:** Short-term supplementation with fish oil FAs seems to increase the accuracy of two-step TST.

**Keywords:** Fish Oils, Polyunsaturated Fatty Acids, Dietary Supplements, Tuberculin Test, Clinical Trial

## 1. Background

Tuberculin skin test (TST) has been widely used to determine any immunological reactivity to mycobacterial antigens (1). Because the health care workers (HCWs) are vulnerable to tuberculosis (TB) infection, they are screened with TST to identify latent TB infection (2, 3) although 10% to 20% of TB patients show negative results on TST (without immunosuppression) (4). Hence, HCWs, who have undergone annual tuberculin skin testing, are recommended to do a two-step testing on initial evaluation, with a second TST 1 week after a negative initial test (1). Tuberculin reactions may decrease or increase in size due to immunologic recall of pre-existing delayed type hypersensitivity to mycobacterial antigens, or new infection (5).

Two-step tuberculin testing is an essential tool for identifying persons with the baseline positive tuberculin test results and allows accurate reporting of subsequent HCW tuberculin conversions (6). HCWs are much more likely to have boosting than conversion, except in case of the initial two-step testing pre-exposure (1). In other words, two-step initial TST may help eliminate nearly 80% of false-positive conversions; nevertheless, interpretation of repeated TST results is difficult because of the nonspecific

variations in test results (7, 8). Some studies demonstrated that some factors are associated with a boost; eg, age, infection with atypical mycobacteria, and previous Bacillus Calmette-Guerin (BCG) vaccination (1).

Animal studies have suggested that a boosted skin test response correlates with the serum immunoglobulin G reactions against a range of mycobacterial antigen proliferative responses (9). However, the effect of immunomodulator supplements on the skin test results is uncertain. The omega-3 fish oil contains both docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The “n-3” fatty acids (FA) that are essential nutrients are known as regulators of inflammation (10). Epidemiological studies have shown the benefits of fish oil in inflammation-associated pathologies (10, 11). There are controversial reports about the effects of fish oil FA on T cell immune function in humans. Some studies found the deficiency of (n-3) FA as a contributing factor to cutaneous anergy, but in other studies DHA supplementation resulted in no effects on delayed-type hypersensitivity (DTH) skin response (12).

Those controversies will raise questions about the effect of fish oil consumption on tuberculin test (a DTH response that shows cutaneous reaction to purified protein derivative (PPD) as a manifestation of cell-mediated immu-

nity). This study aimed at responding to this question and determining the effect of the short duration fish oil FA consumption on two-step TST results in young healthy medical and nursing student volunteers.

## 2. Methods

### 2.1. Study Design, Setting, and Population

In the present study, the entire population was used as the sample. A census is attractive for small populations (eg, 200 or less), eliminates sampling error, and provides data on all the individuals in the population (13). All students from medical and nursing schools of Qazvin University of Medical Sciences (QUMS) ( $n = 213$ ) were invited to participate in this study. Those volunteers who did not have evidence of tuberculosis were enrolled. Exclusion criteria included any type of known allergies, clinical disorder, and consumption of immunosuppressive medications, or steroidal anti-inflammatory drugs. Information on demographics, some anthropometric indices, history of exposure to TB, family size, and smoking were gathered using a questionnaire. This study was registered in the Iranian registry of clinical trials (IRCT), with a registration number of IRCT2015040620882N2.

### 2.2. Intervention and Study Outcomes

Participants were randomly assigned into a conventional diet (control), or fish oil (treatment) group using computerized simple randomization. Computer-generated random-numbers software was used to generate a random allocation sequence. The researcher who was in charge of reading the second TST was blind to group assignment. TST have been carried out at baseline and 1 week after the initial inoculation (in 2 periods between December 1 - 8, 2013, and May 9 - 16, 2014). The second TST was applied for all participants in both groups, except for the 36 dropped outs. The daily intervention in the treatment group consisted of 1 gram fish oil soft gel capsule which contained 180 mg EPA and 120 mg DHA FAs, manufactured by biotech USA International Corporation Ltd. The selected dose is the minimum limit of fish oil supplementation with physiological effects and without any toxicity (14). The capsules were given for 5 consecutive days because according to an expert panel, the probability of continuous consumption of supplements may decrease after the first week.

The outcome of the trial was a change in 2 sequential (primary and secondary) purified protein derivative test (PPD) induration sizes. Six mm or more were considered as a criterion to distinguish increases in reaction size due

to random variation alone from true biologic phenomena (1, 15, 16).

DTH skin test was performed at the baseline and after 1 week. The DTH skin test is an in vivo indicator of specific cell-mediated immune responsiveness by T cells. Tuberculin testers were trained according to standard guidelines (17). All participants were tested with 0.1 mL 5TU-PPD (5 tuberculin units of PPD; Razi institute, Tehran, Iran). The PPD injected intradermally on the volar aspect of the left forearm. The site of injection was marked with a felt marker. All tuberculin solutions were stored in the vaccine carriers at 2 - 6°C over the period of study. The tests were read after approximately 48 hours. The maximum transverse diameter of induration (not erythema) was measured with a ruler for each participant.

### 2.3. Ethical Considerations

The participants freely participated in this clinical trial and could withdraw from the study at any time they wished. The ethics committee of the QUMS approved the study. Written informed consent was obtained from each participant.

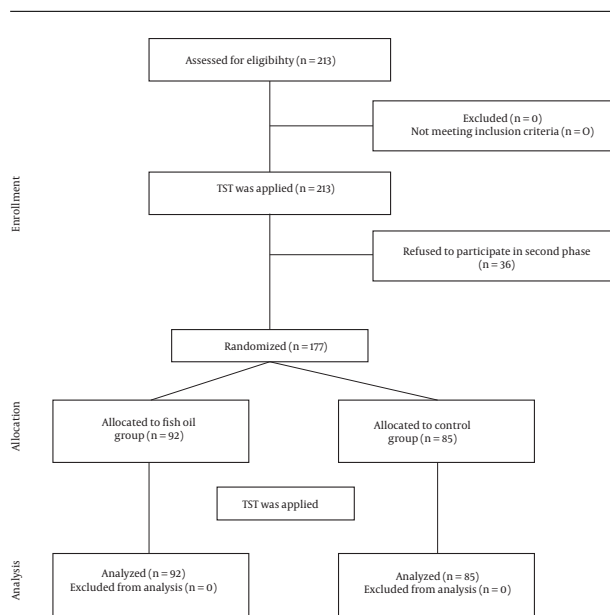
### 2.4. Statistical Analysis

Continuous variables were summarized as means  $\pm$  standard deviation (SD) and categorical variables as frequencies and percentages, unless otherwise stated. The treatment group was compared to the control using Chi-square and Fisher's exact tests for nominal, and student's t test for continuous variables. The association between levels of change in skin test reactivity and exposure was evaluated by Chi-square test. Binary logistic regression analysis was done to assess the effect of fish oil consumption on the secondary induration size status. Moreover, linear regression analysis was used to assess the effect of fish oil consumption on the difference between the secondary and primary induration sizes. Pearson Chi-square test was conducted to examine the relationships. SPSS for Windows, Version 21 (SPSS Inc., Chicago, IL) was used for statistical analyses. P-value of  $< 0.05$  was considered as statistically significant.

## 3. Results

In the present study, 213 students of QUMS were considered for inclusion, 36 (17%) of them refused to receive their second shot. Thus, 177 students, aged 18 - 46 years were randomly allocated into treatment (1 gram fish oil soft gel capsule) and control groups (Figure 1).

The participants had a mean age of  $21.58 \pm 4.50$  years (Range: 18 - 46) and nearly 2/3 (65%) of them were female.

**Figure 1.** Trial Profile

Randomized groups did not show any difference in age, body mass index (BMI), first induration size, family size, smoking status, and gender before the intervention (Table 1).

**Table 1.** Comparing Baseline Characteristics of the Participants Between the Two Groups<sup>a</sup>

Variables	Group		P Value
	Treatment	Control	
Age, y	21.92 ± 3.30	21.20 ± 1.53	0.066 <sup>b</sup>
BMI, kg/m <sup>2</sup>	22.60 ± 3.05	22.57 ± 3.04	0.947 <sup>b</sup>
Family size	4.17 ± 1.43	4.40 ± 1.50	0.305 <sup>b</sup>
First induration size, mm	4.17 ± 4.81	4.52 ± 4.17	0.613 <sup>b</sup>
Male, %	33.7	36.5	0.700 <sup>c</sup>
Smoker, %	6.5	1.2	0.120 <sup>d</sup>

Abbreviations: BMI, Body Mass Index; SD, Standard Deviation.

<sup>a</sup>Value are expressed as Mean ± SD.

<sup>b</sup>Student t test.

<sup>c</sup>Pearson Chi-square.

<sup>d</sup>Fisher's exact test.

All participants had already been vaccinated against TB with BCG vaccine and were determined to be free of any chronic systemic disease. Seven participants had a known history of TB contact. No adverse events were reported in the treatment group during the course of the study. The mean secondary induration was significantly higher in fish oil users than in controls ( $6.68 \pm 0.70$  vs.  $3.61 \pm$

$0.42$ ), as well as the difference between the primary and secondary induration sizes ( $2.51 \pm 0.52$  vs.  $-0.91 \pm 0.34$ ) ( $P < 0.001$ ). The proportion of an increasing size of TST induration in the intervention group was higher (16.3 vs. 1.2%) (Table 2).

**Table 2.** Tuberculin Skin Test Variation in the Two Groups<sup>a</sup>

Induration Level	Group Number		Total
	Treatment	Control	
Significant decrease in size <sup>b</sup>	5 (5.4)	8 (9.4)	13 (7.3)
Unaffected	72 (78.3)	76 (89.4)	148 (83.6)
Significant increase in size <sup>b</sup>	15 (16.3)	1 (1.2)	16 (9.0)
Total	92 (100.0)	85 (100.0)	177 (100.0)

<sup>a</sup>Value are expressed as N. (%).

<sup>b</sup>Six mm or more, (Chi-square, 12.8;  $P < 0.01$ ).

To assess the contributors of induration size change, we entered consumption of fish oil, age, sex, BMI, first induration size, family size, history of TB contact, and smoking into the binary logistic regression model. After entering the method analysis, consumption of fish oil and first induration size remained in the model. Increasing age was another contributor for induration difference (Table 3).

#### 4. Discussion

The present study aimed at providing a better understanding of the previously observed effects associated with fish oil supplementation and examining the impact of fish oil FAs on the immune system function of healthy volunteers. This was one of the few studies reporting the effects of short-term fish oil FAs on cellular immune response of healthy individuals. In our study, fish oil consumption affected the DTH reaction in healthy participants (18, 19).

In our study in the two-step TST of healthy individuals, a significant increase in the size of TST induration was a rare phenomenon (1.2%), but with administration of fish oil, this increased to 16.3%. Our results revealed that the short-term intake of n-3 fatty acid may increase the ability to respond to tuberculin antigen that are in accordance with human trials reporting increased T cell-mediated immune responsiveness (20). Most animal studies (mainly containing a high level of FA or a long-term consumption) have indicated that fish oil FAs suppress the immune responses both in vivo and in vitro (21-24). In addition, in some human reports, high level or long time dietary fish oil impairs lymphocyte responses (12). In our study, a decreasing size of TST induration in the treatment group was not significantly different between the 2 groups (5.4% vs.

**Table 3.** Factors Influencing the Induration Difference (Linear Regression, Method = Enter)

Variables	Standardized Coefficients Beta	P Value	95% CI for B	
			Lower	Upper
First induration size	-0.281	0.004 <sup>a</sup>	-0.346	-0.068
Age	1.758	0.001 <sup>a</sup>	0.159	0.586
Group	-1.064	0.000 <sup>a</sup>	-4.355	-1.917
BMI	-0.497	0.274	-0.281	0.080
Sex	0.384	0.107	-0.226	2.287
Smoking	0.117	0.857	-2.736	3.286
Contact with TB+	-0.345	0.587	-3.729	2.117

Abbreviations: BMI, Body Mass Index; CI, Confidence Interval; TB, Tuberculosis.

<sup>a</sup>Predictors in the Model: first induration size, age and group. Negative values of beta indicate a reverse association

9.4%). The results of our study are different from those of some previously reported studies. Kelley et al. found that supplementation of healthy young men with DHA would result in no effects on DTH skin response (25). However, there are differences in experimental designs and methods between the studies, which may account for those variations.

With increasing age, induration size of TST (resulting from mycobacterial infection) may decline in some people and result in a nonreactive TST (26) although in our study, the high first induration size and the highest increasing size of TST induration belonged to the older students. The most likely explanation for this is “natural boosting”. Mycolyl transferase is present in BCG and exists in all mycobacterial species, thus, allowing the natural boosting of specific immune responses via environmental exposure to even nonpathogenic mycobacteria (27). Evidence of natural boosting has been demonstrated in TB endemic areas (28).

In the present study, increased size of TST induration was observed in a few nonreactive participants (1.2%) in the control group after the second TST. A probable reason is “booster phenomenon” or increased size of the reaction to second test administered 1 week after the primary TST. Similarly, the results of another study indicated a reactivity enhancement in only a minority of serial tests with PPD antigen (1).

This study had some limitations. First, our results were not adequate to definitively confirm an important role of the two-step TST concurrent omega-3 consumption strategies in a different clinical context. Second, we studied a limited population of students in a university; therefore, further large-scale validation studies have to be conducted before recommending or applying this strategy even in a similar setting. Although participants were not blinded to

the type of treatment (fish oil taste), the readout TST results were quantitative and the criteria for a positive response were predefined.

#### 4.1. Conclusions

In summary, age and fish oil consumption were identified as the most important independent contributors of TST induration size in two-step testing. Overall, our results suggest that a short-term supplementation with fish oil FAs may improve the ability of healthy volunteers to respond effectively to antigens of mycobacterium tuberculosis and likely increase the accuracy of two-step TST. Nevertheless, we believe that the more comprehensive studies with different terms and dosages should be carried out to better evaluate the effect of fish oil consumption on two-step TST results. Ideally, a large prospective study is needed to verify and elucidate the association between fish oil consumption and TST size changes.

#### Acknowledgments

The authors wish to acknowledge the assistance of the staff of the clinical skills lab (QUMS) for their help and support and thank all the participants for their dedication to this study. Also, the authors would like to express their gratitude to the Deputy of Research and Technology of QUMS for his support in conducting this study.

#### Footnote

**Conflict of Interest:** The authors declared no competing interests.

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